27. ANALYSIS OF A SAGE (SERIAL ANALYSIS OF GENE EXPRESSION) CATALOGUE OF HUMAN GRANULOSA CELLS

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Mammalian ovarian follicles are composed of an oocyte surrounded by granulosa (or follicle) cells. Granulosa cells are essential for successful oocyte maturation and ovulation. A gene expression profile, or transcriptome, provides a catalogue of the genes expressed by a cell or tissue and the levels of individual transcript expression. In the case of the granulosa cell, its transcriptome should allow insight into granulosa cell function and potentially lead to markers of follicle quality for use in human assisted reproduction such as *in vitro* fertilization (IVF). We used SAGE (serial analysis of gene expression) as a technique to determine the transcriptome of granulosa cells. SAGE relies on the assumption that a 14-base sequence whose position is defined in relation to the 3'-end of any transcript uniquely identifies the gene from which the transcript originated (1). Human granulosa cells were obtained from Otago Fertility Services, Dunedin, New Zealand from 4 adult females, at the time of IVF treatment. After purification, total RNA (5.96 μg) was extracted from these cells using the Qiagen RNeasy kit. The SAGE library was constructed with the Invitrogen I-SAGE kit. Briefly, SAGEtags were extracted from concatemerized ditag sequences using SAGE2000 software (Invitrogen, version B). In total 59 clones were sequenced, yielding 1339 SAGEtags. Selected human SAGE libraries (normal ovary, heart, liver, lung, pancreas and white blood cell) were downloaded from the NIH Sagemap website (ftp.ncbi.nih.gov/pub/sage/seq/) and their relative SAGEtag abundances were compared to that of the human granulosa SAGE library. It was found that human granulosa cells had a unique pattern of gene expression and a number of tags were found that had high abundance in the human granulosa SAGE library, but were absent from other libraries (for example, hydroxysteroid (11-beta) dehydrogenase I and scavenger receptor class B member 1). Those genes that were common to many cell and tissue libraries tended to be structural and house-keeping genes (for example gamma actin). PCR techniques were used to independently validate the SAGE catalogue.

(1) Velculescu, V. E., Zhang, L., Vogelstein, B., Kinzler, K. W. (1995). Serial analysis of gene expression. *Science* **270**, 484–487.

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